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DIAGNOSIS OF GROUP A STREPTOCOCCAL INFECTIONS DIRECTLY FROM THR--ETC(U)
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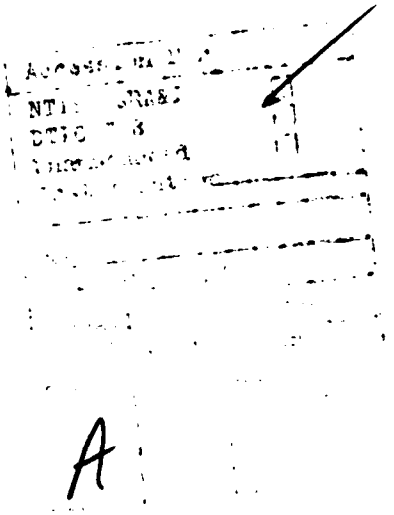
SUMMARY

Streptococcal disease remains a serious problem in our military population today in spite of our modern medical capability. The laboratory identification of streptococci in clinical samples still relies upon culturing the organism on a blood agar plate and further specific identification by immunochemical procedures which can take another 24-48 hrs. The purpose of the present communication is (1) to demonstrate the presence of "free" streptococcal antigen in throat secretions (in this case, a gargle) and (2) identification of streptococcal infection can rapidly be made directly from this antigen without the conventional blood agar culture procedure. While the sample population we report on is relatively small, the results are encouraging and if further studies confirm these preliminary studies, the diagnosis of group A streptococcal disease should become a rapid and simple procedure that could be performed in remote military facilities with minimal laboratory capability as well as in the large medical centers.



INTRODUCTION

The early identification of group A streptococci is essential for selecting appropriate antimicrobial therapy to prevent suppurative complications of streptococcal disease. Because clinical diagnosis of streptococcal pharyngitis is unreliable,[1,5] throat cultures are necessary for the management of the patient. Although beta hemolytic streptococci can be recovered within 18-24 hours from a throat culture, the widely used bacitracin disk susceptibility test to differentiate group A streptococci from other beta hemolytic streptococci requires another 18-24 hours. The more recently described tests using sensitized staphylococci[3] (COAG) (Phadebact, Pharmacia) or the latex test (Streptex, Wellcome) have made precise identification easier but have not reduced the time required for identification. Because of the delay in getting culture results, many physicians either do not take cultures or may, while the patient is in the office, administer antimicrobial therapy "to play it safe" in case the culture later yields beta hemolytic group A streptococci.

Other previously described rapid methods of streptococcal detection are the fluorescent antibody technique[4] and counterimmunoelectrophoresis.[2] However, neither of these techniques have come into widespread use.

A simple technique that would permit identification of streptococci directly from throat secretions would allow immediate application of appropriate antimicrobial therapy in the management of streptococcal infections. It would also be valuable as an aid in the detection of group A streptococci in health surveys. Such a method appears possible using a latex agglutination test. This application of latex particles sensitized with specific antibody (Streptex group A, Wellcome) to detect streptococcal antigen directly from throat gargles is described here.

MATERIALS AND METHODS

Clinical Samples: Approximately 10 ml of PBS or nutrient broth was used as a gargle by individuals reporting to the Marine Corps dispensary with sore throat complaints. A throat swab was taken at the same visit just prior to the gargle. The swab was streaked onto a sheep blood agar plate for streptococcal isolation and identification. Gargle material was cultured on sheep blood agar for group A streptococci and tested for streptococcal group A antigen by latex aggregation as described below.

Latex Test: Latex (Streptex A, Wellcome, Lot #K0271 and K5674) was used as follows: Gargle material was centrifuged at 400 x G for 10 minutes. The clear supernatant was removed and heated in a boiling water bath for 3 minutes. After cooling, five drops (0.25 ml/drop) were transferred to a 10 x 75 mm test tube and 1 drop (.025 ml/drop) of a freshly prepared Trypsin solution was added (0.01% suspension of Difco Trypsin 1:250 Lot #A145356). The mixture was heated at 37°C for 30 minutes. One drop of the trypsinized gargle was mixed with 1 drop of Streptex group A on a microscopic slide. The slide was tilted to and fro for 3-5 minutes. Reading for aggregation was made using a stereomicroscope. For a control, Streptex group B (Lot #K7971 Wellcome) was used in place of Streptex group A.

Sensitivity of latex, COAG and CIE in detecting antigen: Ten fold dilutions of an antigen prepared from a stock strain of group A streptococci was made in PBS. Testing for antigen by the latex, COAG, and CIE methods either followed the manufacturer's directions or were tested as previously described.[2]

RESULTS

An example of the sensitivity of the CIE, COAG and latex tests to detect streptococcal group A antigen is shown in Table 1. The latex test has consistently been the most sensitive of the 3

TABLE I

A comparison between latex, COAG, and the CIE test in detecting group A streptococcal antigen in spiked buffer solutions

	Antigen Dilution									
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}
CIE	+	-	-	-	-	-	-	-	-	-
Latex	+	+	+	+	+	+	+	+	±	±
Staph	+	+	+	-	-	-	-	-	-	-

tests. In testing gargle material for the presence of streptococcal antigen, it was found that the majority of gargles caused spontaneous aggregation of the latex test reagent. Our preliminary studies indicate that trypsin "destroys" the factor(s) responsible for the non-specific aggregation but does not interfere with latex/antigen complexing when antigen is present. In a clinical trial comparing conventional culture and latex aggregation to identify group A streptococcal infection, a total of 53 throat cultures/gargle were collected, 31 were culture positive and 29 were positive by latex (Table II). An example of a positive test result is shown in Figure 1.

TABLE II

Comparison between latex and the conventional method for identifying group A streptococci organisms on antigen from throat gargles and/or throat swabs. Correlation was significant (χ^2=25.6316, p= <0.01)

		Culture results +	Culture results --	Total
Latex results	+	26	3	29
	-	5	19	24
Total		31	22	53

Figure 1 - A typical latex aggregation test using Streptex A reagent (left). Streptex B reagent is used as a neagtive control (right). If Streptex B is positive either there is a dual infection or there has been inadeuate enzyme treatment of the gargle.

DISCUSSION

Testing complex biological secretions for antigen has been troublesome because of non-specific factors which can interfere with test interpretation. With the use of latex in highly contaminated fluids such as the gargle described here, "non specific" aggregation of the latex was a major problem. The substance in gargles causing aggregation of the latex particles is, at this time, unknown. It was resistant to heating in a boiling water bath for at least 5 minutes and has not been dialyzable. Attempts to destroy the aggregating property of the substance with various concentrations of trypsin or pronase B were successful over a broad range of enzyme concentrations: trypsin .002% - .1% and pronase B .01 to 2 mg/ml, (pronase at final concentrations of 12 mg/ml or greater spontaneously aggregated the latex). This suggests a protein nature of the aggregating substance. Our experience with either of these enzymes indicates that their use may be a relatively simple solution to the non-specific aggregation of latex by biological secretions. We have also applied ELISA procedures to identify streptococcal antigen from gargles. While antigen could be detected by these procedures directly from gargles, non-specific reactions were so frequent that test results were difficult to interpret.

The latex aggregation test in this preliminary study compares favorably ($p < 0.01$) with the conventional culture method to identify group A streptococcal infections. If future studies confirm the results of these observations, streptococcal infections could readily be made a relatively simple routine office procedure. The optimal method of acquiring throat samples from young children is yet to be determined.

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The diagnosis of group A streptococcal disease still relies on isolation on sheep blood agar followed by presumptive identification of group A streptococcal strains by using bacitracin sensitivity or the more precise sero-grouping methods such as the Lancefield precipitin test. A technique that would permit rapid identification of streptococcal infections directly from throat secretions would allow immediate appropriate antimicrobial therapy in the management of streptococcal infections. We have been able to identify soluble

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Group A antigen directly from throat gargle by using a latex aggregation test. In a clinical trial using latex (Streptex group A) 53 throat culture/ gargles were taken, 31 were culture positive, and 29 were latex positive for group A streptococcal antigen.